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Studies on the damage potential of the predator *Nesidiocoris tenuis* on tomato plants

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Abstract

Nesidiocoris tenuis (Reuter) (Heteroptera Miridae) is an important natural enemy of whiteflies in Mediterranean field and protected tomato crops. However, this species has been related to the damage induction on tomato plants. Feeding on tomato plants may cause the development of necrotic rings on the stems and flower abortion. This damage has been reported as important in certain cases whereas in other studies there was very low damage evidence. In our study, the damage potential of the predator was evaluated on caged tomato plants. On the plants no prey was present. Various densities of different stages of the predator were released on each plant. These densities were 16 and 32 young nymphs (of the 1st, 2nd or 3rd instar), 16 and 24 large nymphs (4th or 5th instars) and finally 16 and 32 adults. In all cases controls were used. The number of necrotic rings on the stem, and each leaf was recorded after 9, 16 and 23 days of the predators' release in the cages. Damage on the flower clusters was recorded one month after the predator's release. According to the results, the predator inflicted a very small number of necrotic rings on the stems and the flowers and thus this damage was not considered as important. Flower abortion was not observed and the number of rings on the flower petioles was kept at very low levels. The number of flowers developed was similar to that of the control plants. Therefore, our experiments showed that this predator has a low potential to cause damage on tomato stems and flowers even when it occurs at high densities. However, further studies should explore its damage potential after a longer interaction with the plants and under a wider range of temperatures than those evaluated in the present work. In addition, studies on damage records on fruits could highly contribute to a more thorough investigation on the damage potential of this mirid.

Key words: flower abortion, mirid, necrotic rings, polyphaghy.

Introduction

Species of the family Miridae (Rhynchota Heteroptera) show diverse feeding habits that extend from strict phytophagy to zoophagy (Dolling, 1991; Coll and Ruberson, 1998). Between these two extremes there are several species that show omnivorous (zoophytophagous) habits as they can feed both on prey and plant material. Omnivorous species of this family are major natural enemies of several pests such as whiteflies, in solanaceous field and greenhouse crops (Albajes and Alomar, 1999). Besides their beneficial role in pest regulation, some of these species have been associated with damage production of host plants. *Dicyphus tamaninii* Wagner has been reported as causing blemish on tomato fruits when populations of prey species decline (Alomar and Albajes, 1996; Sengonca *et al*., 2003).

Nesidiocoris tenuis (Reuter) is considered as a major natural enemy of insect pests on tomato crops in the Mediterranean region. It commonly colonizes tomato crops and substantially contributes to the control of whitefly and other pests (Malausa and Ehanno, 1988; Arzone *et al.*, 1990; Goula and Alomar, 1994; Tavella *et al*., 1997; Carnero *et al*., 2000; Sanchez *et al*., 2003; Lykouressis *et al.*, unpublished data). Its population trends followed those of whiteflies, showing its potential in biological control (Sanchez, 2008). Laboratory studies proved that it feeds on whiteflies, as well as on eggs and early larval instars of Lepidoptera (Torreno and Magallona, 1994; Carnero *et al*., 2000; Urbaneja *et al*., 2005). This predator feeding solely on a plant diet seems to have little potential to complete development in the absence of prey, although tomato seems to favor its development compared to eggplant and pepper (Urbaneja *et al*., 2005).

Despite its importance in biological control, the status of this predator on tomatoes has not been clarified, as its presence has been related to the creation of necrotic rings on stems, leaves or flower petioles that may reduce the vigour of the plants or cause flower abortion (El Dessouki *et al*., 1976; Vacante and Tropea Garzia, 1994). However, *N. tenuis* adults or nymphs, enclosed on a tomato shoot, initiated the development of necrotic rings on the stem, but the harm was not found significant and these rings were soon disappeared (Arnó *et al*., 2006). However, studies on the damage potential on flowers would be of particular importance (Arnó *et al*., 2006). Similarly, damage due to predator's feeding on the shoots has not been proven important, but flower abortion has been considered as essential (Sanchez, 2008). Thus, further studies are required to qualify more closely the potential of *N. tenuis* to cause different types of damage. Related studies are of high interest for the utilization of the predator in biological control programmes and in development of its appropriate conservation strategies in tomato agroecosystems.

The objective of the current study was to investigate the damage potential of different population densities of *N. tenuis* on the shoots, leaves and flowers of tomato plants. The densities used covered a wide range of high densities that might be occurred under field conditions. The insects were kept on the plants for a long period in order to attain a complete understanding of the damage potential of this predator under realistic conditions.

Materials and methods

The damage potential of the predator *N. tenuis* was evaluated in cage experiments conducted in the campus of the Agricultural University of Athens (AUA).

N. tenuis was reared on tomatoes (cv. Primadonna) that were developed without the use of pesticides. The rearing was initiated from nymphs and adults collected on tomato plants in the area of Trifylia, western Peloponnesus. They were kept in wooden-framed muslin cages (80 x 80 x 70 cm) in an experimental glasshouse of the laboratory of Agricultural Zoology and Entomology in AUA. Eggs of *Ephestia kuehniella* Zeller (Lepidoptera Pyralidae) were provided as prey *ad libitum*. This prey was proved a high quality food resource for the predator (Urbaneja *et al*., 2005).

Tomato plants were transplanted individually in pots of 32 cm diameter. As substrate a mixture of turf:perlite 5:1 was used. At transplantation, the plants bore 4 fully expanded leaves but inflorescences were not developed. The plants had not been treated with any kind of chemical and were kept free from any insect or mite.

Each plant was enclosed in a fine muslin cage (70 cm high) that was appropriately adjusted on each pot. Each cage bore vertically a zipper, of equal length with its height, to facilitate observations inside the cage. The plants were irrigated every 2 days and fertilized in 1 week intervals with 25 ml per plant of a suitable soluble mixture (12-12-17-2-8 in N, P, K, B and Zn). On each plant 2 shoots were retained.

In each cage *N. tenuis* either nymphs of different instars or adults were introduced. All the individuals used in the experiments were collected from the rearing. The densities used per cage were 16 and 32 young nymphs (of the 1^{st}, 2^{nd} or 3^{rd} instar), 16 and 24 large nymphs (of the 4^{th} or 5^{th} instars) and finally 16 and 32 adults of unknown age. Each treatment was replicated 8 times (i.e. 8 tomato plants each in separate cage were infested by the mirid). As controls 8 caged tomato plants without *N. tenuis* were used. The caged plants were kept in the open and they were arranged randomly at distance of about 1m from each other in order to avoid any variation to their growth due to different orientation. All the plants were kept free of any prey so that to force *N. tenuis* to show its phytophagous potential, as phytophagy is expected to be more intense under prey scarcity (Sanchez*,* 2008). Nymphs were included in the evaluation, as they are more likely related to damage production than the adults (Arnó *et al*., 2006).

The predator densities used were selected based on experimental evidence. In greenhouse samplings, the maximum average population density on a single tomato leaf of *N. tenuis* was about 5 individuals (Sanchez, 2008). The peak densities of *N. tenuis* individuals per plant in tomato greenhouse and open field crops were ranged at similar levels (Arnó *et al*., 2006). The maximum average population densities of *N. tenuis* was about 6 individuals on a tomato leaf sample, in the area of western Peloponnesus (Lykouressis *et al*., unpublished data).

The predator's introduction in the cages took place on June 9, 2008. Records of the damage inflicted by *N. tenuis* on the plants were taken on leaves, shoots and flowers. The number of brown necrotic rings around the leaf or leaflet petioles was monitored on all fully expanded leaves of each plant. Records took place on 18, 25 June and 2 July i.e. 9, 16 and 23 days after the predators' release. Damage on each of the 2 shoots that were retained on each plant was recorded on 9, 16 and 23 days after the predators' release. In this case the rings on the top part of each shoot above the first fully expanded leaf were counted. The plants were inspected for flower abortion on all samplings but flower abortion was not recorded.

Damage records on flower clusters took place on 9 July i.e. one month after the predators' release. Then, the flower clusters developed on half of the plants of each treatment were cut off and transferred to the laboratory where they were carefully examined under stereomicroscope for the presence of necrotic rings. On these plants, the total number of flowers developed per plant was also recorded. At this stage, there were also fruits developed on the plants. These fruits were recorded as surrogates of flowers. Thus, the number of fruits was added to the number of flowers present at the time of the record and the result considered as the total number of flowers developed per plant during the course of the experiment.

In addition, one month after the release of the predator in the cages, the number of *N. tenuis* individuals was recorded on those plants used for the flower records, as described above. Discrete measures were taken on each plant for the nymphs (separated in two categories: 1^{st}, 2^{nd} and 3^{rd} instars, and 4^{th} plus 5^{th} instars) and adults.

The temperature and relative humidity data during the course of the experiments were obtained from the Hellenic National Meteorological Service from a station located about 10 km north of the site of the experiments. The data were collected in 3-h intervals.

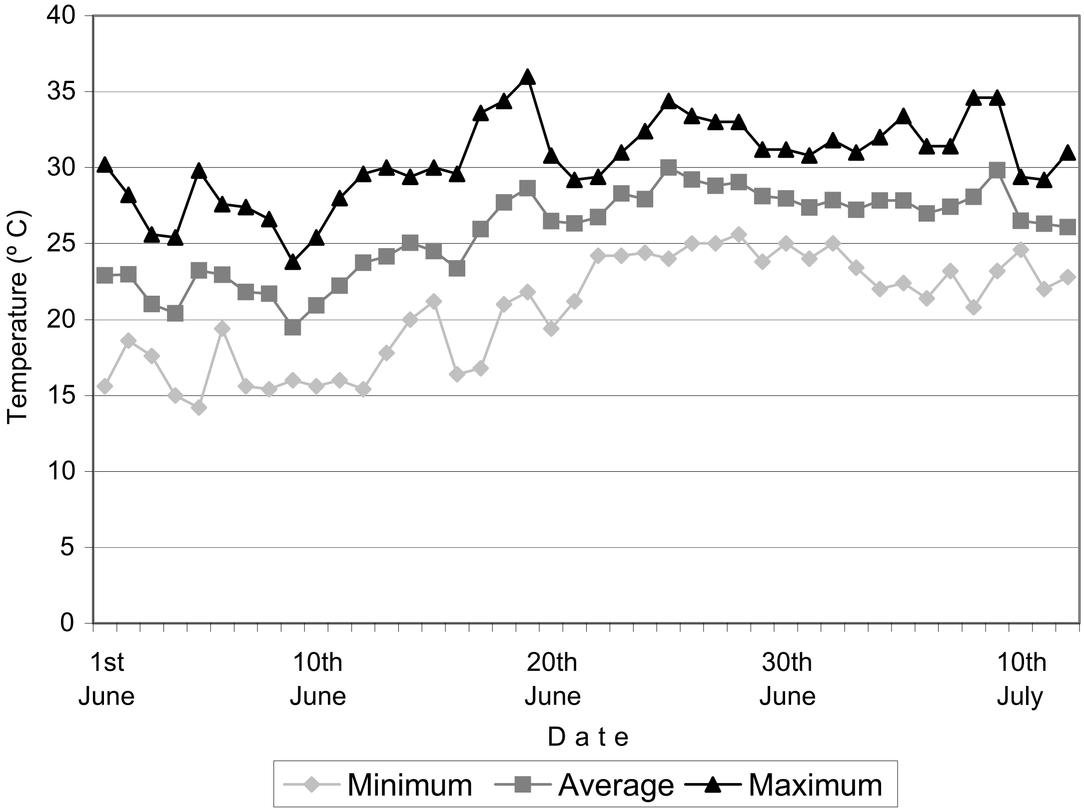
Data of the damage on leaves and on the top part of the shoots were analysed using a repeated measures ANOVA. The within subjects factor was the time (date) of the records and between subjects factor the treatment (i.e. the different densities and stages of the predator). Data on the numbers of *N. tenuis* individuals and flower numbers at the end of the experiments were analysed employing one-way ANOVA with factor the treatment (density levels of *N. tenuis*).

The data on damage records and numbers of *N. tenuis* individuals were square root transformed, whereas flower numbers were $\log_{10}(x)$ transformed prior to the analysis. Analyses were conducted using the statistical package JMP 7.0.1. (SAS Institute 2007).

Results

The temperature and humidity data during the course of the experiments are shown in figure 1.

The damage on tomato leaves was significantly affected by the time and the treatment ($F = 8.20$, d.f. = 1,48, $p < 0.001$ and $F = 7.69$, d.f. = 6,48, $p < 0.001$, respectively). The interaction of these two factors was not significant ($F = 0.88$, d.f. = 6,48, $p > 0.51$). In all cases



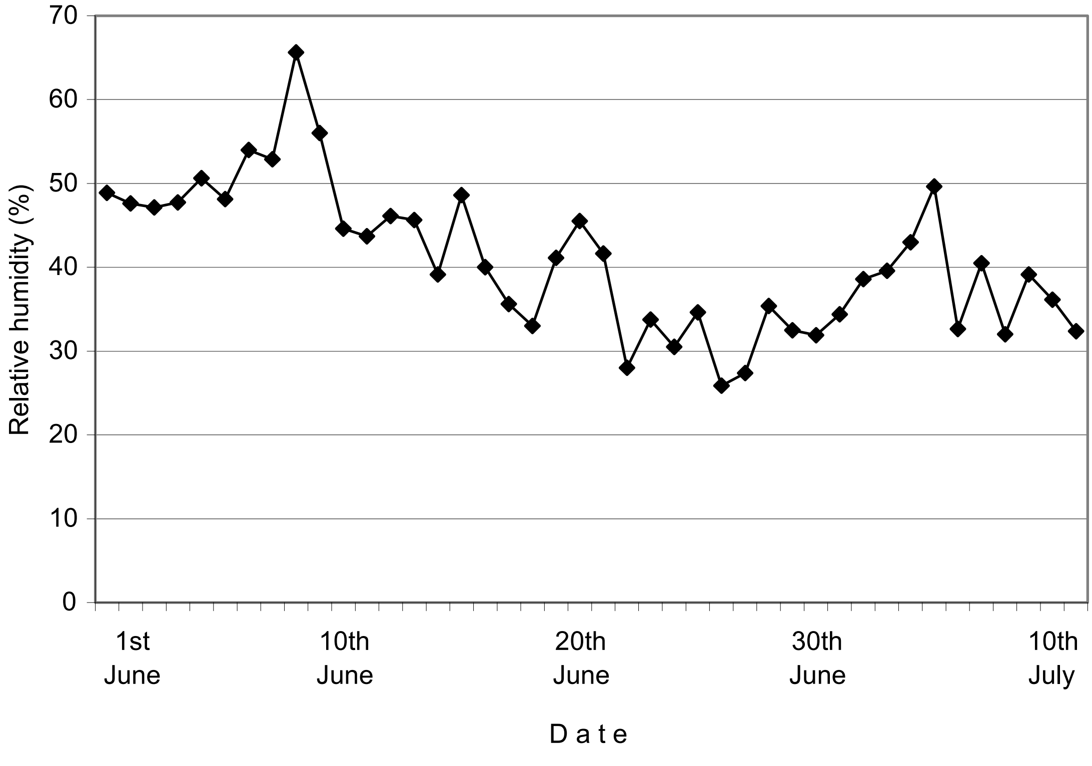


Figure 1. Temperature and relative humidity data during the course of the experiments as obtained by the Hellenic National Meteorological Service.

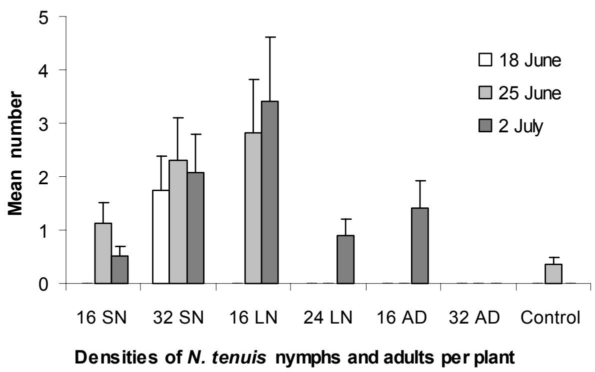


Figure 2. Mean number (± SE) of necrotic rings on all the leaves per tomato plant at different dates, in cages where a single tomato plant had been enclosed along with different densities of *N. tenuis* on 9 June, without prey (SN: 1st to 3rd instar nymphs, LN: 4th and 5th instar nymphs, AD: Adults). Records were taken on 18, 25 June and 2 July i.e. 9, 16 and 23 days after the predators' release.

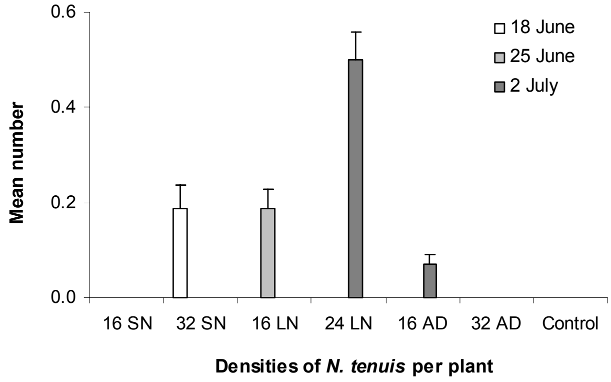


Figure 3. Mean number (± SE) of necrotic rings on the top part of tomato plants at different dates, in cages where a single tomato plant bearing two shoots had been enclosed along with different densities of *N. tenuis* on 9 June, without prey (SN: 1st to 3rd instar nymphs, LN: 4th and 5th instar nymphs, AD: Adults). Records were taken on 18, 25 June and 2 July i.e. 9, 16 and 23 days after the predators' release.

only a small number of necrotic rings were recorded (figure 2). Their frequency on all the leaves of a single plant showed a peak of 3.41 ± 1.21 (mean ± SE).

Damage levels on the top part of the shoots of each plant was significantly influenced by the treatment but not by the sampling occasion (F = 4.23, d.f. = 6,48, p < 0.02 and F = 2.64, d.f. = 1,48, p > 0.12, respectively) and their interaction was significant (F = 5.27, d.f. = 6,48, p < 0.001). Generally their numbers were kept at low levels and showed a peak that reached 0.5 ± 0.06 rings per shoot (figure 3). In most cases, these rings were produced by nymphs whereas adults did not cause such a kind of damage. During the experiments no withering of leaflets or the apex was observed.

The number of necrotic rings on flower clusters is shown in figure 4. This type of damage did not overreached 0.92 ± 0.35 rings per cluster. Its incidence was not affected by the treatment (F = 1.51, d.f. = 6,77, p > 0.18).

The number of flowers developed on the plants was not influenced by the treatment (F = 0.92, d.f. = 6,21, p > 0.49). The maximum number of flowers per plant was 36.5 ± 5.19 (figure 5).

The number of *N. tenuis* individuals present in the cages on 9 July ranged at relatively low levels. The effects of treatment and predator's stage were significant on the number of individuals recorded (F = 3.71, d.f. = 5,72, p < 0.004 and F = 14.94, d.f. = 3,72, p < 0.001) but their interaction was not significant (F = 1.71, d.f. = 15,72, p > 0.07). In the cages that nymphs had been enclosed, mostly nymphs of the 1st and 2nd instar were recorded (figure 6). Their maximum number per plant reached to 18.75 ± 7.61. In the cages where adults had been enclosed, late instar nymphs were prevalent in the population, with their numbers reaching 13.75 ± 4.11 per plant.

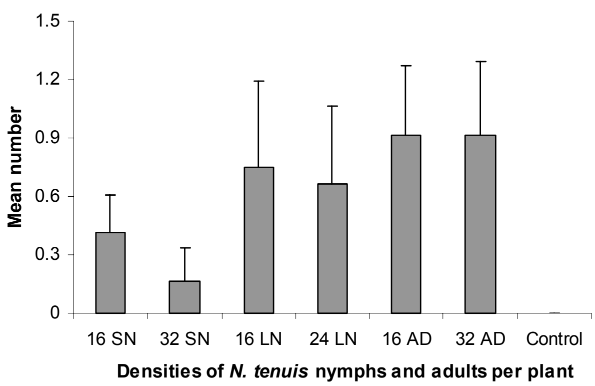


Figure 4. Mean number (± SE) of necrotic rings on each flower cluster of tomato plants on 9 July (i.e. 1 month after the predators' release), in cages where a single tomato plant had been enclosed along with different densities of *N. tenuis* on 9 June, without prey (SN: 1st to 3rd instar nymphs, LN: 4th and 5th instar nymphs, AD: Adults).

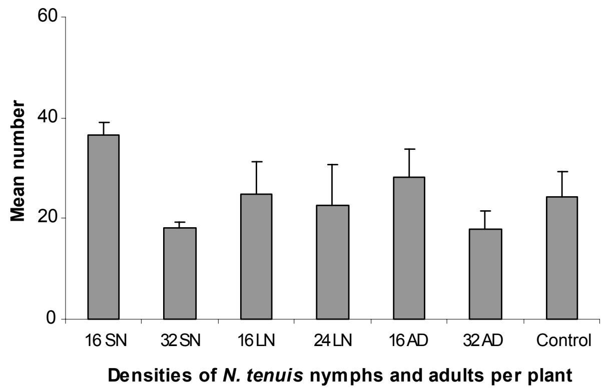


Figure 5. Mean number (± SE) of flowers developed on each tomato plant on 9 July, (i.e. 1 month after the predators' release), in cages where a single tomato plant had been enclosed along with different densities of *N. tenuis* on 9 June, without prey (SN: 1st to 3rd instar nymphs, LN: 4th and 5th instar nymphs, AD: Adults).

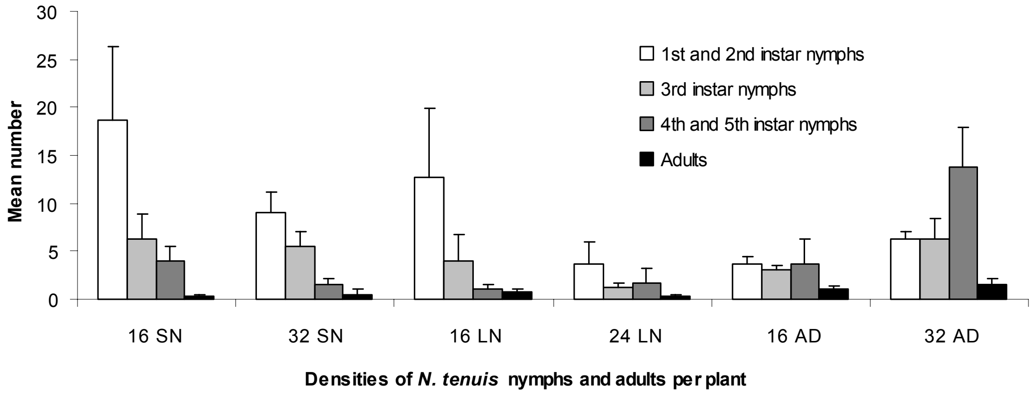


Figure 6. Mean number (± SE) of *N. tenuis* individuals recorded on each tomato plant on 9 July, (i.e. 1 month after the predators' release), in cages where a single tomato plant had been enclosed along with different densities of *N. tenuis* on 9 June, without prey (SN: 1^{st} to 3^{rd} instar nymphs, LN: 4^{th} and 5^{th} instar nymphs, AD: Adults).

Discussion

Our results showed a low damage potential of *N. tenuis* on tomato shoots or leaves, as the number of necrotic rings was kept at very low levels. Furthermore, flower abortion was not observed and flowers developed as normal.

According to Arnó *et al.* (2006) 10 or 20 adults enclosed per caged side shoot of tomato plants for 48h caused a number of necrotic rings that reached about 0.5 rings per shoot; 20 nymphs caused about 3 rings per shoot. The damage production of *N. tenuis* on tomato plants was also investigated by Sanchez (2008). In tomato greenhouses, the population densities of *N. tenuis* were related to the damage incidence on the plants. It was found that the highest damage incidence (5 necrotic rings on the upper 20 cm of the plant shoot) was produced when about 3 *N. tenuis* individuals per leaf occurred in a period when the number of the whiteflies had been low. In laboratory trials, 5 *N. tenuis* females per caged tomato plant caused the infliction of 1-2.6 necrotic rings in a period of 1 week, in prey absence. Therefore, the experimental evidence collected in the current study as well as that of the previously mentioned studies on the damage potential of *N. tenuis* on tomato stems or leaves, supports the conclusion that it does not seem to be significant, for the respective conditions used.

In addition, the damage potential was found to be mostly attributed to the presence of nymphs with the adults to be much less effective in production of necrotic rings as it was also reported by Arnó *et al.* (2006).

According to our results, flower abortion was not observed and flowers developed as normal. Arnó *et al.* (2006) mentioned that although flower abortion was not closely monitored, flower clusters developed normally. However, it has been reported that *N. tenuis* could cause the abortion of flowers. This abortion may reach a high percentage such as 50% in a period when *N. tenuis* occurred at densities of about 2 individuals per plant and the whitefly numbers were almost negligible in tomato greenhouses (Sanchez, 2008). Evidence for damage infliction has been derived also in field experiments (Vacante and Tropea Garzia, 1994).

These different conclusions on flower abortion among studies might be due to the different experimental material used, such as the tomato varieties. On the other hand, they also may indicate that there is a variation in the phytophagous ability of *N. tenuis* populations originating from different geographical areas. However, factors such as temperature or the physiological state of the plant could be likely included in the sources of the results' variation on flower abortion regardless to the presence of *N. tenuis* on the plants. As stated by Sanchez (2008) the number of necrotic rings was closely correlated with temperature increase from 20 to 30 ºC. However, in our study average temperature was mostly kept at levels lower than 30 ºC (figure 1) and thus, further studies should be conducted for the evaluation of temperature and likely other abiotic factors, on flower abortion by *N. tenuis*.

The low ability of *N. tenuis* to survive when solely phytophagous could be involved in the low damage potential. It has been proven that none of the nymphs developed to the 4^{th} instar, whereas they survived for a total period of 15.6 days at 25 ºC, tested without prey availability on tomato leaves (Urbaneja *et al*., 2005). In our study, *N. tenuis* numbers were before long decreased, however there was a potential to sustain population with a higher success rate compared to what should be expected based on the results of Urbaneja *et al.* (2005). These differences might be due to the use of different plant substrates (plants vs leaves) but also show a variation in the phytophagous habits of *N. tenuis* populations derived from different regions.

In conclusion, *N. tenuis* seems to have a low potential in the damage production on tomato leaves, shoots and flowers. Therefore, it could be considered as a beneficial predator at least when occurs at densities similar to those used in the study. However, further studies should explore its damage potential after a longer interaction with the plants than those evaluated in the present work. In addition, damage records on fruits could highly contribute to a more thorough investigation on the damage potential of this mirid.

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